Safety evaluation of commonly used Chinese herbal medicines during pregnancy in mice

Chi Chiu Wang1,2,*, Lu Li1,†, Ling Yin Tang1,†, and Ping Chung Leung3

1Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, 1st Floor, Block E, Prince of Wales Hospital, Shatin, New Territories, Hong Kong 2School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong 3Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

*Correspondence address. Tel: +852-2632-2810; Fax: +852-2636-0008; Email: ccwang@cuhk.edu.hk

Submitted on February 16, 2012; resubmitted on April 12, 2012; accepted on April 25, 2012

BACKGROUND: It is unclear how safe the use of Chinese herbal medicine is during pregnancy and if the herbal medicines do any harm to pregnancy, embryo–fetal development and prenatal and post-natal growth. A large-scale preclinical study was conducted to detect the adverse effects of Chinese herbal medicines during pregnancy.

METHODS: Twenty of the most commonly used Chinese herbal medicines prescribed for pregnancy were selected and the crude extract was administered to pregnant mice at clinical doses during five different gestational stages, namely post-implantation, gastrulation, organogenesis, maturation and whole gestation periods. Maternal effects on side effects, weight loss, litter reduction, implantation failure and fetal resorption and perinatal effects on growth restriction, developmental delay, congenital malformations and post-natal mortality were determined.

RESULTS: Adverse pregnancy outcomes were commonly observed after maternal exposure to the herbal medicines, particularly during early pregnancy. Major events included maternal and perinatal mortality were recorded. Maternal weight gain, embryo growth and post-natal weight gain were significantly decreased. Fetal resorption and skeletal malformations were significantly increased.

CONCLUSIONS: Reproductive toxicity of Chinese herbal medicines commonly used during pregnancy was identified in mice. Caution should be taken in the clinical use of herbal medicines during pregnancy.

Key words: Chinese medicines / herbal medicines / pregnancy / safety / reproductive toxicity

Introduction

Traditional Chinese medicine is currently well accepted as a mainstream of medical care throughout East Asia and is considered a complementary or alternative medicine in the Western world (Basics, 2010). Chinese herbal medicines are common name for Chinese Materia Medica, which have therapeutic properties for medical treatment and healing (Read, 1976). It is considered as a primary modality of internal medicine in traditional Chinese medicine (Holland, 2000). For centuries, Chinese herbal medicines have been widely used to relieve many symptoms and to treat complications during pregnancy (Flaws, 2005). The prevalence of the use of Chinese herbal medicines in China and South-East Asia countries are high, 78.7% in Japan (Mantani, 2003), 55% in Hong Kong (Ong et al., 2005), 32% in Mainland China (Wang et al., 1995) and 24% in Taiwan (Chuang et al., 2009). Chinese herbal medicines have been used to prevent spontaneous abortion and preterm labor, and to manage the common cold, low back pain, placenta previa, fetal growth restriction and other obstetric problems (Fu, 1978).

Some Chinese herbal medicines are commonly used during pregnancy and they are claimed to be ‘safe’ and ‘effective’ (Lei et al., 1995). Unlike those pharmaceutical drugs not recommended for use during pregnancy because of known adverse or teratogenic effects in animal studies and/or clinical studies, Chinese herbal medicines are prescribed based on personal experience in clinical practices; however, there are no sufficient statistics or scientific data regarding the undesirable maternal and perinatal consequences of their use. Until now, it is unclear how safe the use of Chinese herbal medicines is during pregnancy and if there are any adverse effects to pregnancy, embryo–fetal development and prenatal and post-natal growth.

† These authors contributed equally to this work.

© The Author 2012. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved. For Permissions, please email: journals.permissions@oup.com
The strategy for testing herbal medicines in humans has not yet been established. Before clinical studies can be undertaken in humans, the very first step in developing guidelines on the assessment of quality, efficacy and safety of herbal medicines is to test in animals. The aim of the present study was to determine the safety of Chinese herbal medicines in pregnancy by screening Chinese herbal medicines commonly used during pregnancy. The selected Chinese herbal medicines were administered to pregnant mice in order to determine the adverse maternal and perinatal outcomes. This is the first comprehensive preclinical study on a large scale to test the safety of Chinese herbal medicines during pregnancy in mice.

Materials and Methods

Setting and subjects

The studies were conducted according to US Food and Drug Administration Guidelines in Detection of Toxicity to Reproduction for Medicinal Products in animals (2006-ICH-SSA). It is recommended by the regulatory bodies in European Union, Japan, China and Taiwan and applicable to medical and biological products (ICH, 1994). The Institute of Cancer Research (ICR) mice were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong. The protocol was approved (06-073-MIS) and guidelines were followed for the use and care of all laboratory animals as set in the university.

Chinese medicines

To determine the safety of Chinese herbal medicines in pregnancy, only those Chinese medicines commonly used during pregnancy were tested. Based on our literature research (Li, 2011), the top 20 most commonly used Chinese medicines during pregnancy were selected from the clinical studies of Chinese herbal medicines for the treatment of threatened spontaneous abortion (Table I, see also Supplementary data, Appendix and Supplementary data, Table S1). According to Chinese Pharmacopoeia (National Pharmacopeia Committee, 2010), those Chinese medicines of animal origin with known allergic effects, prohibited in other countries, from heavy metal with potential risk of poisoning, classified as toxic, ‘not recommended’ or contraindicated in pregnancy were excluded. Donkey-hide Glue, a gelatin extract from a dry glue prepared from the hide of a rare black-skinned donkey; Motherwort Herb, from which a lethal case in human has been reported (Jia, 1989) and Baical Skullcap Root, from which a similar animal study had been extensively studied and reported by Tian et al. (2009), were not included. In total, 17 Chinese medicine extracts prepared from standardized decoction methods for clinical use using state-of-art concentration technologies in Good Manufacturing Practice standards were purchased from PuraPharm, Hong Kong. The quality of each medicine was controlled by recognized organoleptic authentications according to guidelines of the Chinese Pharmacopoeia. Molecular authentication by PCR, direct amplification of length polymorphism, and/or amplified fragment length polymorphisms were employed to amplify the unique DNA sequences specific to the herbal species in order to confirm the genetic composition for each taxon. Chemical authentications by thin-layer chromatography and/or high-performance liquid chromatography were conducted to separate chemical contents of the test medicine in order to confirm the quality and quantity of the active chemical constitutions in each medicine and to avoid any pesticide, mineral and other biological contaminations. Only authentication verified medicines were used for study. Each Chinese medicine extract was weighed and dissolved in distilled H2O before administration.

Interventions

Individual medicine was tested one by one to investigate the potential effects of each medicine on mice. Pregnant mice at defined gestational stages and developmental windows, namely post-implantation, gastrulation, organogenesis, maturation and whole gestation periods, were tested (Supplementary data, Table S2). This covered examination periods for the reproductive effects of the test Chinese medicines on early pregnancy, embryo–fetal development, prenatal and post-natal growth and whole gestation. Each animal was weighed and the test Chinese medicine extract was administered orally via the gastric tube. The test dosages for mice were calculated from the clinical dosage for human (Table I, see also Supplementary data, Table S1) based on the recommended dose formula for Chinese herbal medicines: 

\[ d_A = d_B \times \left( \frac{R_B}{R_A} \right) \times \left( \frac{W_A}{W_B} \right)^2 \]  

(You, 2007).

\[ d_A \] stands for the known dose for animal A (in mg/kg), while \( d_B \) stands for the unknown dose for animal B (in mg/kg), \( R_A \) and \( R_B \) are the body size factors, i.e., 59 for mouse, 90 for rat, 93 for rabbit and 100 for human, while \( W_A \) and \( W_B \) are the weights of animals A and B. The final doses were corrected by the preparation ratio of the raw herbal medicine to final medicinal extract (3:1 in grams). The pregnant mice were randomized to receive a single bolus dose of either single, double or triple clinical dose once a day. For each test medicine, 5–10 animals per dose group were treated. The treatment was divided into post-implantation period (Stage I, feeding from gestational days E3–E6), gastrulation period (Stage II, E6–E8), organogenesis period (Stage III, E8–E15), maturation period (Stage IV, E15–delivery) and whole gestational period (Stage V, E0–delivery). In the maturation and whole gestational periods, all pups were followed up until post-natal day P28. Vehicle with distilled H2O only was included as the negative sham control.

Main outcome measures

At the end of intervention, maternal and perinatal outcomes were studied (Supplementary data, Table S2). For post-implantation, gastrulation and organogenesis periods, implantation loss was determined by early and late fetal resorption of the disintegration and assimilation of the dead embryos in uterus. Fetal size parameters determined by the crown-rump length and head length of the embryos were measured and the number of somites were counted under dissecting microscope. The placental size was determined by the average of largest and perpendicular diameters of each placenta. For maturation and whole gestational periods, the duration of pregnancy was determined from the day of conception plug to the day of parturition. Prenatal and post-natal mortalities were determined by stillbirth and death after birth, respectively. Major congenital malformations included external and visceral structural anomalies at necropsy. Maternal weight gain during pregnancy and after delivery and the litter size were recorded in all periods. The neonatal weight of each pup was recorded weekly after birth until weaning.

Statistical analysis

The sample size was calculated based on our previous study (Li et al., 2011). The anticipated difference in an adverse pregnancy outcome, such as decreased fetal growth parameters, was 0.04 and the anticipated standard deviation was 0.01; therefore, at least five animals per treatment group was required for type I error 0.01 with power 99%. Comparisons were made within each individual medicine only. Homogeneity of variances were tested by Bartlett’s examination. Homogenous data were analyzed using ANOVA, while non-homogenous data were analyzed using non-parametric tests, such as the Kruskal–Wallis test and Dunn’s test. The number of resorptions, dead fetuses and litter-based percentage values were analyzed by non-parametric methods. Litter incidence was
<table>
<thead>
<tr>
<th>No.</th>
<th>Hz</th>
<th>English names (Chinese)</th>
<th>Therapeutic action[b]</th>
<th>Clinical dose[c]</th>
<th>Other applications[d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>Largehead Atractylodes Rhizoma (Baizhu)</td>
<td>Invigorate the spleen and replenish Qi; eliminate damp</td>
<td>12.7</td>
<td>Loss of appetite, abdominal distension and diarrhea, dizziness and palpitation, edema, sweating</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>Chinese Dodder Seed (Tustz I)</td>
<td>Replenish and tonify the liver and the kidney</td>
<td>21.8</td>
<td>Impotence, seminal emission, dripping of urine after urination, enuresis, frequent urination, aching and weakness of the loins and knees, blurred vision and tinnitus, diarrhea, vitiligo</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>Himalayan Teasel Root (Xuduân)</td>
<td>Replenish the liver and the kidney; strengthen tendons and bones</td>
<td>15.3</td>
<td>Aching and weakness of the loins and knees, rheumatic arthralgia, abnormal uterine bleeding or menorrhagia, traumatic injuries</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>Donkey-hide Glue (Ejiao)</td>
<td>Nourish yin and blood; relieve dryness</td>
<td>6.4</td>
<td>Anemia, palpitiation and muscle weakness, insomnia numbness and tremors, dry cough, hemoptysis, hematemesis, hematuria, menorrhagia</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>Chinese Taxillus Twig (Săngishêng)</td>
<td>Reinforce the liver and the kidney; strengthen tendons and bones</td>
<td>17.9</td>
<td>Rheumatic or rheumatoid arthralgia with aching and weakness of the lower back and knees, abnormal uterine bleeding, menorrhagia, hypertension</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>Liquorice Root (Gàncâo)</td>
<td>Reinforce the spleen; replenish Qi; remove heat and counteract toxicity</td>
<td>6.2</td>
<td>Lassitude and weakness, cardiac palpitation and shortness of breath, cough with phlegm, spasmodic pain, carbuncles and sores, reduce the toxic or drastic actions of other drugs</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>Milkvetch Root (Huángqí)</td>
<td>Reinforce Qi</td>
<td>22.9</td>
<td>Lack of strength, anorexia and loose stools, chronic diarrhea, prolapse of the rectum, hemafacia, sweating, edema, abscesses difficult to burst or heal, anemia, wasting thirst, uterine bleeding, albuminuria in chronic nephritis, diabetes</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>White Peony Root (Báishāo)</td>
<td>Subdue hyperactivity of liver; nourish blood</td>
<td>15.5</td>
<td>Headache and dizziness, costal and abdominal pain, spasmatic pain of limbs, anemia, menstrual disorders, sweating</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>Chinese Angelica (Dânggūl)</td>
<td>Enrich the blood; activate circulation</td>
<td>10.1</td>
<td>Anemia with dizziness and palpitation, menstrual disorders, amenorrhea, dysmenorrhea, constipation, rheumatic arthralgia, traumatic injuries, carbuncles, boils and sores</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>Baical Skullcap Root (Huângqín)</td>
<td>Remove damp heat; quench fire and counteract toxicity</td>
<td>10.1</td>
<td>Chest distress, nausea and vomiting in epidemic febrile diseases, abdominal distension, acute dysentery or jaundice, cough, high fever with dire thirst, hematemesis and epistaxis, carbuncles and sores</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>Eucommia Bark (Dûzhǒng)</td>
<td>Tonify the liver and the kidney; strengthen tendons and bones</td>
<td>14.7</td>
<td>Lumbago and lack of strength, hypertension</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>Steamed Rehmannia Root (Shûdihuâng)</td>
<td>Nourish yin; replenish the blood; reinforce the essence and marrow</td>
<td>21.6</td>
<td>Aching and weakness of the loins and knees, hectic fever and bone-steaming, night sweating, seminal emission, thirst, anemia with sallow complexion, palpitation, menoxenia, abnormal uterine bleeding, dizziness, tinnitus, premature senility</td>
</tr>
<tr>
<td>13</td>
<td>19</td>
<td>Rehmannia Root (Dângshên)</td>
<td>Reinforce Qi; invigorate spleen and lung</td>
<td>18.8</td>
<td>Shortness of breath, cough, palpitation, anorexia, loose stools, wasting-thirst</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>Common/Wingde Yan Rhizoma (Shânyâo)</td>
<td>Replenish the spleen and the stomach; promote fluid secretion and benefit lung; strengthen kidney</td>
<td>18.2</td>
<td>Anorexia and chronic diarrhea, cough and dyspnea, seminal emission, excessive leucorrhoea, frequent urination or diabetes</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>Villous Amomrum Fruit (Șaharâni)</td>
<td>Eliminate damp; warm spleen</td>
<td>6.5</td>
<td>Epigastric stuffiness and loss at appetite, vomiting and diarrhea, morning sickness</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>Raw Rehmannia Root (Shângdîhuâng)</td>
<td>Remove heat; promote production of blood fluids; reduce heat in blood</td>
<td>21.6</td>
<td>Thirst, skin eruptions and maculation, hematemesis, epistaxis, sore throat</td>
</tr>
<tr>
<td>17</td>
<td>12</td>
<td>Szechuan Lovage Rhizoma (Chûnxîng)</td>
<td>Promote blood and Qi; dispel wind</td>
<td>7.4</td>
<td>Menstrual disorders, amenorrhea, dysmenorrhea, abdominal pain, pricking pain in the chest and costal regions, swelling and pain, headache, rheumatic arthralgia</td>
</tr>
</tbody>
</table>

Continued
analyzed using the chi-square test for all groups followed by Fisher’s two-tailed test with the Bonferroni correction for each treatment group versus the negative control.

**Results**

Detailed outcomes of each individual Chinese medicine are summarized in Supplementary data, Tables S3–S7. Reproductive effects of the Chinese medicines within a particular gestational period were compared.

**Benefits**

Beneficial effects of the Chinese medicines to pregnancy defined as the enhancement of maternal and fetal conditions during pregnancy were not commonly observed in our study. The common favorable outcomes, including enhanced embryonic growth and development as indicated by significantly increased embryo lengths and/or somite numbers and increased litter size, were found in organogenesis period with Largehead Atractylodes Rhizome, Liquorice Root, Chinese Angelica, Eucommia Bark, Steamed Rehmannia Root and Pilose Asiabell Root (Supplementary data, Table S5). In addition, significant increased maternal weight gain, litter size and post-natal weight gain during the whole gestational period was observed in the White Peony Root (Supplementary data, Table S7). Similar benefits in maternal weight gain, litter size and post-natal weight gain were occasionally recorded in other medicines, but were not consistent in higher doses or correlated with other measures within the gestational periods. The beneficial effects were not dose-dependent, and there were no significant correlations between the doses and the outcomes.

**Major events**

Maternal and post-natal mortality were observed during the intervention. Post-partum maternal mortality occurred in the maturation period with Largehead Atractylodes, Chinese Dodder Seed, Liquorice Root, White Peony Root, Szechuan Lovage Rhizome and Tangerine Peel (Supplementary data, Table S7). Only Himalayan Teasel Root and Chinese Taxillus Twig significantly increased the maternal death before delivery in whole gestational period and the incidences seemed to be dose dependent; 0% in a single clinical dose, 20–22% in a double clinical dose, then 50–60% in a triple clinical dose. The maternal death was not associated with other adverse pregnancy outcomes, including decreased maternal weight gain and reduced litter size. No obvious gross pathological changes were found in autopsy examination.

Perinatal mortality was significantly increased in White Peony Root, Raw Rehmanna Root and Szechuan Lovage Rhizome during the maturation period (Supplementary data, Table S6) and whole gestational period (Supplementary data, Table S7). The incidences were varied amongst the medicines, 28–67% in White Peony Root, 20–25% in Raw Rehmanna Root and 25% in Szechuan Lovage Rhizome. The perinatal death presented as either stillbirth at delivery, early death (within 7 days) or late death (within 28 days) in the post-natal period. No obvious dose-dependent effects were observed. No major gross pathological changes were found in autopsy examination.

**Other harms**

Harmful effects of the Chinese medicines during pregnancy were very common. It involved both early and late gestational periods. The common adverse outcomes in early gestations included increased resorption rate and decreased embryonic growth and development, whereas the common adverse outcomes in late gestations included increased post-natal mortality and congenital malformation rate, and decreased post-natal weight gain. Milkvetch Root, Chinese Angelica and Chinese Mugwort Leaf have more adverse outcomes in early gestations, whilst Largehead Atractylodes Rhizome and Chinese Dodder Seed have more adverse outcomes in late gestations. The other medicines have similar adverse outcomes in either early or late gestations. Only Chinese Dodder Seed, Himalayan Teasel Root and Szechuan Lovage Rhizome showed a dose-dependent effect, i.e. higher dose results in more adverse outcomes.

In post-implantation period (Supplementary data, Table S3), Chinese Taxillus Twig, Liquorice Root, Villous Amomrum Fruit and Chinese Mugwort Leaf significantly reduced maternal weight gain and increased early fetal resorption rate. In the gastrulation period
and Raw Rehmannia Root significantly increased the post-natal weight gain. In the whole gestation period (Supplementary data, Table S6), Chinese Taxillus Twig and Wingde Yam Rhizoma significantly decreased the embryonic growth and development. In organogenesis period (Supplementary data, Table S5), adverse outcomes were not common, but some medicines were with a high fetal resorption rate (Fig. 1). In the maturation period (Supplementary data, Table S6), Chinese Taxillus Twig and Wingde Yam Rhizoma significantly shorten the pregnancy duration, increased congenital malformation and post-natal mortality rate, and reduced post-natal weight gain. In the whole gestation period (Supplementary data, Table S7), Largehead Atractylodes Rhizome, Chinese Dodder Seed, and Raw Rehmannia Root significantly increased the post-natal mortality rate.

Malformations

Congenital malformations, mainly skeletal limb abnormalities, were observed in the organogenesis (Supplementary data, Table S5), maturation (Supplementary data, Table S6) and whole gestational periods (Supplementary data, Table S7). Major limb abnormalities were identified in Largehead Atractylodes Rhizome and Baical Skullcap Root. Minor limb abnormalities, mainly polydactyly and oligodactyly, were observed in Chinese Dodder Seed, Steamed Rehmannia, Szechuan Lovage Rhizome, and Tangerine Peel. Upper limb abnormalities were more common than lower limb abnormalities. The anomalies in Largehead Atractylodes Rhizome included extensions and abduction of both upper and lower limbs. Posterior shoulder and hip dislocations, ankle dislocation and caudal regression were detected by skeleton staining. Shortening of radius and the absence of ulna and first phalangeal bones were confirmed by computer tomography examination (Li et al., 2021). Polydactyly as a very thin appendage beneath the litter finger was commonly found in more than half of the Chinese medicines tested during the organogenesis period (Fig. 2). The incidences were significantly increased in Chinese Dodder Seed (15–35%), Himalayan Teasel Root (8–9%), Liquorice Root (16–21%), Pilose Asiabell Root (7–17%), Wingde Yan Rhizome (9–16%), Villous Amomrum Fruit (7–12%), Szechuan Lovage Rhizome (9–16%), Chinese Mugwort Leaf (14–28%) and Tangerine Peel (7–26%). Oligodactyly was also observed during the organogenesis and maturation periods (Fig. 3). The incidences of oligodactyly were significantly increased in Steamed Rehmannia Root (10%), Szechuan Lovage Rhizome (22%) and Tangerine Peel (7–13%), while digital arrest was observed in Steamed Rehmannia Root (2.8%, Fig. 3).

Discussion

Herbal medicines are generally regarded by the public and some health-care providers as safe and effective (Marcus and Snodgrass, 2005). Despite the lack of scientific basis for the safety claims, vast numbers of pregnant women use it to maintain good health and reduce the need of medication (Westfall, 2001; Ong et al., 2005). Although therapeutic effects of Chinese herbal medicines for complicated pregnancy has been shown in our latest meta-analysis (Li et al., 2012), evidences of adverse events in the use of the medicines during pregnancy are very limited. Up to date, still no regulations have been established for monitoring or controlling the clinical use of herbal medicines during pregnancy. This is the first preclinical study to screen the most commonly used Chinese medicines during pregnancy in animals. The results showed that adverse pregnancy outcomes were very common, suggesting Chinese medicines could do harm during pregnancy. Maternal weight gain was not beneficial from the medicines; however, maternal blood biochemistry, lipid and hormonal profiles, and liver and renal functions were not examined during and after the intervention. Only few medicines enhanced the growth of the developing embryos. It indicates that beneficial effects of Chinese medicines for pregnancy could be rather minimal. Similar beneficial effects have been demonstrated in a Chinese herbal preparation, which contained Chinese Angelica, Eucommia Bark and Steamed Rehmannia Root as in our animal study (Yang et al., 2001). No further detailed investigation has been done so far. However, these may not be clinically relevant because it may associate with the adverse pregnancy outcomes due to macrosomia by promoting fetal growth and multiple pregnancy by increasing the litter size. In contrary, the test medicines in our study resulted in many adverse pregnancy outcomes. Major events included maternal and perinatal deaths. Others included fetal resorption, growth restriction, developmental delay and congenital malformations. Amongst all, Largehead Atractylodes Rhizome was the most harmful Chinese medicines during pregnancy, whilst Liquorice Root was the most safe Chinese medicines during pregnancy. From clinical studies, adverse maternal and perinatal outcomes of Chinese medicines for treatment of threatened spontaneous abortion during early pregnancy were also identified. Preterm labour rupture of membranes, prematurity and associated neonatal mortality were recorded (Cui, 1998; He, 1997; Zhou, 1997; Luo et al., 2007). In our animal study, shorter pregnancy duration, increased fetal resorption and stillbirth and early post-natal loss caused by Chinese Taxillus Twig may relate to the preterm delivery and the perinatal mortality when a herbal formula Shou Tai Pill has been used (He, 1997; Cui, 1998; Zhou, 2006).

Birth defects involving the limb development are quite common, concerning about 1 per 1000 live births (Kliegman et al., 2006). It may be due to cancer, genetic diseases and chromosomal abnormalities, improper position in the womb, infections during pregnancy, injury during birth, malnutrition, metabolic disorders and use of certain drugs during pregnancy (Canale, 2003). In a clinical study of Chinese herbal medicines for threatened spontaneous abortion, ~0.9% congenital malformation rate was reported. Unfortunately, the details of the malformation were not specified (Chou, 2002). In Chou’s study, herbal formulae Shou Tai Pill and Wu Zi Decotion were also used. Both preparations contained Chinese Taxillus Twig and Chinese Dodder Seed, which may be associated with the congenital limb anomalies observed in our animal study. In our study, congenital limb anomalies commonly occurred. According to Swanson’s classifications (Swanson et al., 1983), failure of formation and duplication could be the two main limb deformity mechanisms induced by the Chinese medicines. In Largehead Atractylodes Rhizome, longitudinal postaxial arrest was observed as absence of the ulna bone with minimal carpal coalitions and thumb hypoplasia (Li et al., 2011). In Steamed Rehmannia Root, longitudinal central arrest was observed as the absence of metacarpals within the central portion of the hand. In other medicines, transverse arrest at phalangeal levels might result in oligodactyly. On the other hand, post-axis polydactyly was commonly observed. A case of VACTERL (Vertebral anomalies,
**Figure 1** Fetal resorption in maternal exposure to Chinese medicines during the organogenesis period E8–E15 in mice. Ten Chinese medicines induced late fetal resorption at E15 are shown. Scale bar represents 1 mm.

**Figure 2** Congenital polydactyly deformities in maternal exposure to Chinese medicines during the organogenesis period E8–E15 in mice. (Left panels) Twelve Chinese medicines induced unilateral or bilateral polydactyly appendage (open arrows) at E15 are shown. Scale bar represents 1 mm. (Right upper panel) Vehicle control with normal digit (closed arrow) at E15 is shown. Scale bar represents 1 mm. (Right lower panels) Safranin O cartilage staining showed the presence of cartilage (red stains) and mucin (blue stains) in the polydactyly appendage (open arrow) in the study group, but not in the normal digits (closed arrow) in the control group. Magnification 50X.
Anal atresia, Cardiovascular anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal/Radial anomalies and Limb defects) association presented with unilateral fetal limb defects, and a case of recurrent fetal short-limbed chondrodysplasia were associated with maternal exposure of herbal medicines for treatment of morning sickness and fertility during pregnancy (Syed et al., 1990; Adesiyun et al., 2007). However, the details of the medicines are not available for further interpretation. The causative effects of Chinese herbal medicines in the skeletal malformation and its underlying mechanism requires further in-depth investigations.

Unlike Western herbalism, Chinese medicines include many animal materials and even mineraloid remedies as well as medicinal herbs (Zheng, 2005). In addition, most Chinese medicines are formulated and individualized. A typical formula may contain 3–25 Chinese medicines. As each Chinese medicine has its own pharmaceutical property and potential drug–drug interaction, the application of formulated and individualized medication enhance the therapeutic actions of some herbs and collaborate all the herbs to balance disharmony of each individual for treatment. However, the adverse effects and toxicity of Chinese medicines may vary in different combination, preparation

Figure 3 Congenital oligodactyly deformities in maternal exposure to Chinese medicines during the organogenesis period E8–E15 and the maturation period E15–P0 in mice. (Left panels) Control vehicles resulted in normal digits (closed arrows; E8–E15: blue for right upper limbs; E15–P0: blue for left upper limbs, green for right upper limbs and red for left lower limbs), while the indexed Chinese medicines induced oligodactyly and arrested digits (open blue arrows) at E15 (A) and P5–P7 (B). (Right panels) Alcian Blue–Alizarin Red skeleton staining shows the missing developing digits at E15 (unossified bone as blue stains) in the oligodactyly induced by Tangerine Peel and digital arrest induced by Steamed Rehmannia Root; and the missing developed digits (open blue arrows) and shorten ulnar (open yellow arrow) at P28 in oligodactyly induced by Szechuan Lovage Rhizome (absence of the fifth phalanx) and Tangerine Peel (absence of the first and fifth phalanxes), but not in the normal digits in the control group (closed blue arrow). Scale bar represents 1 mm (A) and 1 cm (B).
and individuals. These increase the difficulty to study which Chinese medicine in the formulae attributes to the specific adverse effects. Furthermore, each herbal medicine may contain many different active components responsible for therapeutic effects, and also for the adverse effects. Detailed studies of the active components are necessary to understand the underlying pharmacological and molecular mechanism potential for the reproductive toxicity. Nevertheless, in our study specific Chinese medicines and their effects on fertility, sexual maturity and conception in males and females were not studied. Fertility, sexual maturity and conception are also considered important reproductive functions. Further in-depth studies are required to have complete picture of the reproductive toxicity of Chinese medicines. Although many teratogenic effects of pharmaceuticals in human were discovered in animals, the uncertainty of safety of Chinese medicines during pregnancy requires clinical studies to confirm its relevance to humans.

In conclusion, potential reproductive toxicity of some commonly used Chinese herbal medicines in pregnant animals was identified in mice. The effects occurred within clinical doses. Special cautions should be taken in clinical use of the herbal medicines during pregnancy.

Acknowledgements

We especially thank Dr Clara Lau and her team from the Institute of Chinese Medicine, the Chinese University of Hong Kong, for the technical advices in Chinese medicines; Laboratory Animal Services Center, the Chinese University of Hong Kong and Prince of Wales Hospital for the technical supports in animal mating and care; and Dr Flora Tong from Department of Obstetrics and Gynaecology, the Chinese University of Hong Kong, for the translation assistance.

Authors’ roles

C.C.W. conceived the project, designed the experiments, obtained research grant and draft the manuscript. L.L. and L.Y.T. performed experiments, acquired the data and analyzed the data. P.C.L. advised and supervised the study, obtained research grant and approved the final manuscript.

Funding

This work was supported by Health and Health Services Research Fund, Food and Health Bureau, Hong Kong Special Administration Region (06070511).

Conflict of interest

None declared.

References


He YP. Shou Tai pill for threatened miscarriage. Guangzhou J Chin Med College 1997;14:84–86 [Chinese].


Luo HT, Zhao SP, Yuan HK. 58 cases of ultrasound observations on threatened abortions with PPS. J Shanxi College Trad Chin Med 2007;30:26 [Chinese].


You SL. *Fu Chan Ke Shi Yan Dong Wu Xue*. Beijing; Chinese Medicine Publisher of China, 2007,7–8 [Chinese].

Zhou Y. Clinical observation on 305 cases of threatened miscarriage treated by integrated traditional Chinese and Western medicine. *Pract J Integr Chin Mod Med* 1997;10:1614 [Chinese].